VII.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY, UNDER THE DIRECTION OF E. L. MARK, XXXIX.

ON SOME LAWS OF CLEAVAGE IN LIMAX.

A PRELIMINARY NOTICE.

By C. A. Kofoid.

Communicated by E. L. Mark, January 10, 1894.

The following is a statement of the results obtained from the study of cleavage in Limax, and of the literature of cell lineage in other invertebrates. It is desirable to confirm my results by a study of cleavage in other forms before the publication of my final paper, and it has therefore seemed best not to defer the presentation of the conclusions to which I have arrived.

A few words in regard to the usage of terms will be necessary. The egg is regarded as having the animal pole uppermost, and the terms *right* and *left*, *upper* and *lower*, are used as resident in the egg itself. Or, to express it in another way, a miniature observer is imagined as placed in the principal (vertical) axis of the egg, with his head at the animal pole, facing the part or parts of the egg under discussion, and the terms *right* and *left*, *upper* and *lower*, are used as determined by this observer. By "a generation of cells" is meant all those cells which are removed from the ovum by the same number of cell divisions, regardless of the time of appearance or position of such cells, i. e. the word is used in its literal sense. This is not the usage of Fol ('75) or Blochmann ('81), who employ the term in its literal sense with reference to the blastomeres through the four-cell stage, but thereafter use it to designate successive sets of four micromeres, naming them in the order of their appearance in time.

As is well known, cells cleave in sets of fours throughout the spiral period of cleavage. The cleavage of the individual cells of the set may be synchronous or successive, and the cleavage of

any given set may or may not coincide with that of the other sets of the same generation; but whatever the modifications, each cell in its origin bears a close relation to three other cells, and these sets of four related cells of co-ordinate origin will be called *quartets.* During spiral cleavage the egg is made up of a number of superposed quartets, and it may be compared to a house of as many stories, each story representing a single quartet of four cells.

The regions of the cleaving egg occupied by the four blastomeres of the four-cell stage and their derivatives during the spiral period will be called *quadrants,* and the four primitive blastomeres and their respective derivatives will be designated by the letters *a, b, c, d,* taken in the order in which the hands of a clock move. In Nereis and Umbrella, these designate the left anterior, right anterior, right posterior, and left posterior quadrants respectively.

The term *spiral* will be used to indicate the divergence immediately after cleavage of the centre of the nucleus of the UPPER one of two daughter cells from the vertical plane passing through the corresponding portion of the LOWER cell and the vertical axis of the egg. The spiral will be a right spiral when the divergence is toward the right (as defined above), and a left one when the divergence is toward the left. Or, using Blochmann's ('81) comparison to the hands of a clock, when the egg is observed from the animal pole the spiral is a "right" one if the divergence of the upper cell is in the direction of the motion of the hands of the clock, a "left" spiral if the divergence is opposite the motion of the hands of the clock. By this method of nomenclature all those spirals whose spindles stand in similar positions with reference to the vertical axis are given the same name.

It should be noted in this connection, that this divergence, or apparent shifting of cells, in Limax at least, is the result of the obliquity of the plane of division, and is predetermined by the *position of the spindle.* This fact, whatever may be the cause of the particular position of the spindle, is the immediate basis of the phenomenon termed the "spiral." The position of the spindle primarily determines the position of the daughter cells, though mechanical environment may secondarily modify that position.

The nomenclature of spirals as followed by Blochmann ('81), Lang ('84), Wilson ('92), Heymons ('93), and others, presents no

constant basis of reference. Not only have these authors named homologous spirals differently, as Lang ('84, p. 325) and Heymons ('93, p. 256), but no one of those named except possibly Lang has used the same method of naming *all* the spirals discussed. In some cases the lower cell of a pair of daughter cells is regarded as the fixed one, in other cases the upper cell is so regarded; or, to express it differently, in some cases peripheral cells are regarded as fixed, in other cases axial ones. In general it seems to have been the custom to consider the larger of the daughter cells as fixed, and the budding smaller cell as the movable one. Two reasons may be cited for the employment of the relative size of the daughter cells as a basis for the nomenclature of the spiral. (1) The larger cell occupies more nearly the position of the mother cell, and it is therefore natural to regard the smaller cell as the movable one. (2) In the first spiral the larger cells (macromeres) are *basal*, and the micromeres upon them are therefore regarded as the movable cells, and this basis adopted in the first spiral is suggested for other spirals. Though this reference of the spiral to the relative size of the cell may furnish a logical basis for nomenclature of spirals where cleavage is *unequal*, it cannot furnish one for those spirals, or eggs, in which cleavage is *equal*. Nor has this basis when once adopted been consistently followed in every case, — as, for example, in Wilson's paper on Nereis ('92). On page 391 he says: "A careful study of the embryo through these changes shows that all of the cell divisions conform to the spiral type. . . . It is also easily seen in the divisions of the secondary micromeres (a^2, b^2, c^2, X). Each of them divides somewhat obliquely (cf. Figs. 25, 26, 33) so that one of the cells lies somewhat lower than the other, and in most cases the lower cell is obviously smaller than the upper. The difference in size is very great in the case of X and x', but is much less in the case of the others ($a^{2.1}$, $a^{2.2}$, Fig. 33). (In the specimen shown in Figs. 25, 26, on the other hand, there is no appreciable difference in size, but I have never seen a case in which the upper cell is the smaller.) If this group of cells be followed around the embryo from right to left (against the hands of a watch), the upper (larger) cell always comes first; i. e. the first division of the second group of micromeres takes place in a left-handed spiral, *like the second division of the first set of micromeres.*"

There is no escape from the conclusion that in this case the

cell of reference is the *larger* one, as it was in the case of the "third and fourth cleavages" (cf. Wilson, pp. 387 and 388). That it is the "larger" rather than the "upper" cell, will be seen when in Wilson's Fig. 21 (reproduced in outline in my Plate I. Fig. 5) we apply to the spirals foreshadowed in the spindles of the cells d^1, X ($= d^2$), and D, the method employed by him in naming this spiral (a^2, b^2, c^2, X). Following around the embryo from right to left the "upper" cell (indicated by the upper end of the spindle) comes first in all three cases, but the spirals are *not all given the same name.* The "larger" cell comes first in d^1 and X, and the spirals are called left-handed spirals. The smaller cell comes first in D, and the spiral is called a right-handed spiral (p. 391). — Let me call attention, in passing, to the fact that, in the system of nomenclature I have proposed, the three spirals above referred to would be given the same name. They would *all* be called right spirals, and in this similarity of name would be recognized the similarity of the position of spindles, and the fact that in passing from right to left the *upper cell* always comes first. The basis on which my system rests is not the varying *size* of the cells, but the more fundamental factor of *position.*—Up to this point in cleavage Wilson has consistently used his system of nomenclature, but upon the next page (p. 392), in discussing the third division of the primary micromeres a^1, b^1, c^1, d^1, resulting in the formation of the cells a^1, b^1, c^1, d^1, and the rosette cells $a^{1.3}$, $b^{1.3}$, $c^{1.3}$, $d^{1.3}$, he abandons the larger cell as the basis of reference, as will be seen in the following quotation: "The four primary micromeres (a^1, b^1, c^1, d^1) bud forth four small cells at their inner angles (at the upper pole) which arrange themselves in a very regular apical rosette, the cells of which alternate with the central micromeres (Figs. 27, 28, etc.). The position of the spindles is the same as in the first division of a^1, b^1, c^1, d^1; i. e. the division follows a right-handed spiral, but the character of the division is very different since the smaller cells are formed at the central instead of the peripheral angles of the cells (i. e. towards instead of away from the vertical axis of the embryo)." In this case the fact that "the position of the spindles is the same" is cited as a basis on which the spiral is named. In my Fig. 6, Plate I., is reproduced in outline Fig. 25, Plate XV., of Wilson's paper. If we apply to the cells c^1 and $c^{1.3}$ the test mentioned by Wilson on page 391, and name the spiral according to the size of the cell that "comes first," we must call it, not a right-hand, but a left-

hand spiral. This is the only case in which the similarity of the position of the spindles is recognized as a factor in the nomenclature of spirals. By this change in the basis of nomenclature he has recognized the interesting fact that there exists in the three successive divisions of the primary micromeres (a^1, b^1, c^1, d^1) an alternation in the direction of the spirals.

This alternation, as described and named by him, is *independent* of that which exists in the first three divisions of the macromeres. It belongs to an entirely separate system, and its relation to successive generations of cells is neither suggested nor discussed.

Not only does the system of nomenclature based on the size of the cells fail to furnish a logical basis for cases of equal cleavage, but it also fails to furnish such a basis for the comparison of cleavage in different forms, for the cells in which the yolk is lodged in the progress of cleavage are by no means homologous cells in different species (cf. Nereis, Neritina, and Umbrella). It therefore seems to me very much more logical to base the nomenclature of spirals upon constant spatial relations than upon the relative sizes of the daughter cells, which are inconstant, or upon the apparent greater shifting of one of the daughter cells referred to *no constant plane.*

The student of cell lineage finds his task much complicated by the various systems of nomenclature already employed, and it seems a pity to introduce still another to add to the confusion. But as all systems heretofore employed fail to recognize the fundamental importance of sucessive *generations of cells*, and as these are the basis of my treatment of the subject, it has been impossible to adopt any of the existing systems. At the same time, it is believed that the system proposed, resting as it does upon generations of cells, is adapted to all forms of spiral and radial cleavage, and will furnish a satisfactory and convenient means of comparison in these cases. Its applicability to spiral cleavage will now be discussed more fully.

Each individual cell of an egg in spiral cleavage can be traced back to one of the four blastomeres *a*, *b*, *c*, *d*, i. e. it belongs to a definite quadrant. It also belongs to a definite quartet or "story" of the egg; it is likewise removed from the ovum by a definite number of cell divisions, i. e. belongs to a definite generation.

Any system of nomenclature involving these three factors will both localize the cell and outline its ancestry. *Each cell of the*

spiral period of cleavage may therefore be designated by three characters: (1) *a letter, as a, b, c, d, indicating the quadrant;* (2) *a first exponent indicating the generation, as* $a^{4.}$, $a^{5.}$, *etc.;* (3) *a second exponent indicating the quartet or story, as* $a^{4.1}$, $a^{4.2}$, $a^{7.16}$, *etc.* Generations are numbered starting with the ovum as the first generation. The number of cells doubles with each succeeding generation, and after the third generation, i. e. after the four-cell stage or the first quartet, the number of quartets is also doubled. The quartets or stories are numbered *from the vegetative toward the animal pole.* Thus in the eight-cell stage the lower quartet is designated by the exponent 1, and the upper quartet by the exponent 2. This principle is followed in the nomenclature of all quartets. During the period of spiral cleavage, the two daughter cells (or quartets) resulting from the cleavage of any given cell (or quartet) never lie in the same plane, and the *lower* cell (or quartet) of the two is always designated by an *odd* exponent and the upper by an *even* one. Thus, when $a^{5.3}$ (— $d^{5.3}$) divides, the resulting cells (quartets) are $a^{6.5}$ (— $d^{6.5}$) and $a^{6.6}$ (— $d^{6.6}$), the latter being nearer the animal pole. The second exponent of the upper daughter cell is always twice the corresponding exponent of the mother cell, and that of the lower cell twice less one. The quartets are thus designated as though all the quartets of their generation were *actually* present, a condition rarely realized in later generations; however, these quartets are potentially present, being represented by their ancestors or descendants, and no confusion need arise over this point.

The following is a scheme of the nomenclature through the sixth generation : —

First.	Second.	Third.	Fourth.	Fifth.	Sixth.
$\overline{abcd}^1$	$\overline{ab}^2$ $\overline{cd}^2$	a^3 b^3 c^3 d^3	$a^{4.2} — d^{4.2}$ $a^{4.1} — d^{4.1}$	$a^{5.4} — d^{5.4}$ $a^{5.3} — d^{5.3}$ $a^{5.2} — d^{5.2}$ $a^{5.1} — d^{5.1}$	$a^{6.8} — d^{6.8}$ $a^{6.7} — d^{6.7}$ $a^{6.6} — d^{6.6}$ $a^{6.5} — d^{6.5}$ $a^{6.4} — d^{6.4}$ $a^{6.3} — d^{6.3}$ $a^{6.2} — d^{6.2}$ $a^{6.1} — d^{6.1}$
1 cell.	2 cells.	4 cells.	8 cells.	16 cells.	32 cells.

The advantages of this system are as follows. (1) It does not involve the confusion of designating two or more different cells by the same characters, as is the case when a daughter cell is given the same designation that the mother cell had; e. g. when the macromere A divides, and the derivatives are named A and a^1 and the same designation is employed for the basal cell in the succeeding divisions. (2) Never more than two exponents need be employed, and practically the limit of three figures is not exceeded. We thus avoid the cumbersome and confusing exponents which characterize the late periods of cleavage in other systems; e. g. $a''_{1.2.1}$ of Heymons ('93, p. 259, Taf. XV. Fig. 20) becomes $a^{9.25}$. (3) The designation affords some clue to the relative position of the cell and the quartet to which it belongs. Thus the quartet with the second exponent 1 is always at the vegetative pole of the egg. The apical quartet is always designated by one of the even numbers 2, 4, 8, 16, 32, etc. The exponents of any cell give a hint as to the designation of the adjoining cells. Thus, $b^{6.8}$ of Figure 3 (Plate I.) lies in contact with $a^{5.3}$ and $b^{5.3}$ and $b^{6.7}$. (4) The derivation of a cell is implied in its designation in every case. Thus, $b^{6.8}$ is derived from the cell b of the fifth generation and fourth quartet, i. e. from $b^{5.4}$, $b^{5.4}$ from $b^{4.2}$, and $b^{4.2}$ from b^3. The mother cell of any given cell is always designated (1) by the same letter, (2) by the first exponent of the daughter cell less one, (3) by one half of the second exponent when that exponent is even, or by one half the sum of the second exponent and one when it is odd. Thus, $b^{6.7}$ and $b^{6.8}$ are derived from $b^{5.4}$. In like manner, the designation of the mother cell determines that of the daughter cells; for example, when $b^{4.2}$ divides, the daughter cells are always designated as $b^{5.3}$ and $b^{5.4}$, *whatever their position and relation to other cells or quartets.* In typical cleavage they will be members of the third and fourth quartets from the vegetative pole. This typical condition is, however, sometimes modified by delayed cleavage, or by the distribution of the yolk.

This system of nomenclature may also be extended to the cleavage of the bilateral period, and to the radial type of cleavage. In the case of cleavage in a plane parallel to the equator, the upper and lower daughter cells may be designated as in the spiral period and type of cleavage. In meridional cleavages the *right* derivative may be designated by the *even* exponent and the *left* by the *odd* in the case of *even* generations, and the reverse

in the case of *odd* generations. Macromeres when present may be designated by capital letters, or any special quartet, as the "primary micromeres" may be distinguished by special forms of type without change of letter or exponents, or subordinate dichotomous systems may be introduced for protoblasts and their progeny.

My work upon Limax is as yet incomplete, but it is probable that at least through the thirty-six-cell stage the cleavage of Limax is identical, blastomere for blastomere, spiral for spiral, with that of Nereis. Beyond that stage I have not followed the cleavage. In the eggs of *Limax agrestis* the yolk is almost equally distributed, and cleavage is almost equal. Macromeres in the etymological sense do not exist after the eight-cell stage, and it is with difficulty that the poles of the egg can be distinguished by the size of the blastomeres after the sixteen-cell stage.

The discussion in Limax will be limited to the generations from the third to the sixth, inclusive.

Third Generation. The cells (a, b, c, d) of the four-cell stage present the typical arrangement of the furrows at the vegetative and animal poles, and the spiral, as indicated by the obliquity of the spindles, must be called according to my nomenclature a *left* spiral, as Heymons ('93, p. 249) has called the similar spiral in Umbrella.

The *Fourth Generation* is reached in the eight-cell stage by the formation of four macromeres ($a^{4.1}$, $b^{4.1}$, $c^{4.1}$, $d^{4.1}$), and four micromeres ($a^{4.2}$, $b^{4.2}$, $c^{4.2}$, $d^{4.2}$), Plate I. Fig. 1. Each micromere as a result of the obliquity of the spindle lies above and to the right of the macromere which has a cognate origin with it, and the spiral is therefore a *right* spiral.

Fifth Generation. The two quartets of the preceding generation divide at about the same time, giving rise to the sixteen-cell stage composed of the four quartets $a^{5.1}$– $d^{5.1}$, $a^{5.2}$– $d^{5.2}$, $a^{5.3}$– $d^{5.3}$, and $a^{5.4}$– $d^{5.4}$. An inspection of Plate I. Fig. 1, shows that the nuclear conditions of the first quartet are slightly in advance of those of the second quartet. Consequently in Limax the twelve-cell stage is abbreviated almost to obliteration, the egg passing from the eight- to the sixteen-cell stage without the intervention of a pronounced twelve-cell stage. This lateral view of the egg also shows that all of the spindles stand in a similar position with

reference to the vertical axis, i. e. the direction of their obliquity is the same, so that if they should be spread out in a plane parallel with the vertical axis of the egg they would be approximately parallel to one another. The left aster is in every case the upper one; and at the end of division the upper derivative will lie to the left of the lower one, not to the right, as in the preceding and in the following generation. The division of the cells will take place in a plane approximately at right angles to that of the preceding division, and approximately parallel to the one preceding that. *This alternation of the direction of the spindles and planes of division in successive generations* is a phenomenon independent of any system of nomenclature of "spirals." It is a factor, however, which in my opinion should be given weight in any system of nomenclature. If we give to the spiral of the second quartet the designation hitherto universally applied to it, we must call it a *right* spiral, and thus ignore the factor of alternation, for that of the first quartet and same generation is called a left spiral. If, on the other hand, we recognize this similarity in the direction of the obliquity of the spindles of both quartets of this generation, we should give their spirals the same name. This is accomplished by referring the nomenclature of the spiral to the basis suggested in the preceding pages, i. e. by regarding the lower of the two daughter cells as fixed, the upper one as movable or diverging. In the case of the two dividing quartets under discussion the *upper* asters of both quartets are the *left* asters, and consequently of the daughter cells the *upper* is the left; i. e. the direction of the divergence or rotation is toward the left, or opposite the direction in which the hands of a watch move, and the spirals in BOTH quartets should be called LEFT spirals.

The *Sixth Generation* is produced by the division of the four quartets of the fifth generation, $a^{5.1}$– $d^{5.1}$, $a^{5.2}$– $d^{5.2}$, $a^{5.3}$– $d^{5.3}$, and $a^{5.4}$– $d^{5.4}$, which results in the formation of the eight quartets $a^{6.1}$– $d^{6.1}$, $a^{6.2}$– $d^{6.2}$, $a^{6.3}$– $d^{6.3}$, $a^{6.4}$– $d^{6.4}$, $a^{6.5}$– $d^{6.5}$, $a^{6.6}$– $d^{6.6}$, $a^{6.7}$– $d^{6.7}$, and $a^{6.8}$– $d^{6.8}$.

These divisions are not synchronous, however, neither are they all accomplished before the divisions resulting in the succeeding (seventh) generation begin; for from this time on in the history of the egg successive generations of cells overlap one another, so that we find in the egg at the same time cells belonging to two or more generations. In every case the cell is designated as

though all the other cells of that quartet, and all other quartets of that generation, were actually present. Thus in Plate I. Fig. 3, the cell $c^{6.7}$ is the only one of its quartet actually formed, and only six of the eight quartets of the sixth generation have as yet arisen. The missing cells and quartets are of course represented in their ancestors of the fifth generation. The divisions of the quartets resulting in the sixth generation will be discussed in the order of their occurrence.

First Quartet. The cells $a^{5.1}$–$d^{5.1}$ divide, forming $a^{6.1}$–$d^{6.1}$ and $a^{6.2}$–$d^{6.2}$ (Plate I. Figs. 2, 4). The cells of the latter (second exponent $= 2$) lie above and to the right of those of the former (second exponent $= 1$) with which they are associated (Plate I. Fig. 2). Therefore the spiral is a *right* spiral. In this generation, as in the preceding, it will be observed that the basal quartet is the first to divide.

Second Quartet. The cells of the quartet $a^{5.2}$– $d^{5.2}$ divide, forming $a^{6.3}$– $d^{6.3}$ and $a^{6.4}$– $d^{6.4}$ (Plate I. Figs. 2, 3, 4), and, as in the preceding quartet, every cell with an even exponent lies above and to the right of the one which was cognate with it, and which has an odd exponent. Therefore this spiral is also a *right* spiral. This division follows immediately upon that of the preceding (first) quartet, and results in the twenty-four-cell stage of Figure 2 (Plate I.). In this stage the embryo is composed of sixteen cells of the sixth generation and eight of the fifth.

The *Fourth Quartet* ($a^{5.4}$– $d^{5.4}$) divides, forming $a^{6.7}$– $d^{6.7}$ and $a^{6.8}$– $d^{6.8}$, Plate I. Fig. 3. Here, as in the other divisions resulting in the sixth generation, the upper derivatives lie to the right of the corresponding lower derivative, and the spiral is a *right* spiral. It is a matter of importance to note that the quartet $a^{5.4}$–$d^{5.4}$, *whose cells are larger than those of its sister quartet* $a^{5.3}$–$d^{5.3}$, divides before the latter quartet does. Plate I. Fig. 3.

Third Quartet. The division of $a^{5.3}$–$d^{5.3}$ results in forming $a^{6.5}$–$d^{6.5}$ and $a^{6.6}$–$d^{6.6}$. This, like the other three, produces a *right* spiral. The division of this third quartet is begun before that of the fourth quartet is completed; likewise, before the completion of the division of this last quartet of the sixth generation, that resulting in the seventh generation begins, and this so far as followed has left spirals. Thus we see that in the cleavage of Limax there exists *an alternation in the direction of the spirals in successive generations.* The spirals of the *even* generations are right spirals, and those of the odd generations are left spirals.

Of the generations discussed, the fourth and sixth have right, the third and fifth have left spirals. This alternation of spirals apparently rests upon the more fundamental and wide-spread tendency of the spindle to take a position at right angles to that of the spindle of the previous division. This tendency can be traced from the last maturation spindle of the ovum throughout the spiral period and in some cases far into the bilateral period of cleavage, as in the development of the mesoderm in Umbrella (Heymons '93). The spiral period of cleavage presents only slight mechanical impediment to the realization of this tendency, which therefore expresses itself here in the ALTERNATION of spirals.

The system of nomenclature here employed for Limax makes it possible to correlate the two hitherto independent systems of alternation, that of the spirals of the macromeres and that of the micromeres, so often noted by writers on cell lineage (Wilson '92, pp. 378, 391, 439, and '93, p. 600). The alternation is now reduced to a single system, based on generations, which harmonizes all of the cleavages of spiral character.

The law of alternation of spirals in successive generations is, I believe, applicable to all forms of spiral cleavage. At the present time we have two extensive papers of a recent date discussing cell lineage. That of Wilson ('92) carries the discussion through the fifty-eight-cell stage of Nereis, and that of Heymons ('93) through the ninety-one-cell stage of Umbrella. In both of these cases the law of alternation of spirals applies *without a single exception, if the spirals are named on the basis proposed in this paper.* According to Wilson ('92, p. 439), Conklin ('91, '92) has found in the cleavage of Crepidula a close resemblance to that of Nereis. Lillie ('93) has found a corresponding agreement in the cleavage of Unio. The law of alternation of spirals will then apply to Crepidula and Unio in so far as they conform to the cleavage of Nereis. In Nereis, Umbrella, and Limax we have forms presenting diverse conditions of development. We have one annelid, and two mollusks of widely separated genera, one land and two marine forms. In Nereis and Umbrella there is a large amount of yolk of a different nature and distribution in the two forms. They also present free larval stages, with different degrees of precocious development. In Limax there is very little food-yolk, and cleavage is almost equal.

The free larval stage is suppressed and the spiral period of cleavage persists for a long time, the fundaments of the organs appearing comparatively late in development. In the matter of the envelopes of the egg, the three forms present very diverse conditions; yet in all these widely differing forms we find no break in the regular alternation of spirals in successive generations as I have defined them. Previous to the publication of Wilson's paper ('92) on Nereis, which has given such an impetus to the study of cytogeny, the results of a number of investigations appeared dealing more or less fully with the phenomena of spiral cleavage. Neither confirmation nor contradiction of an alternation of spirals implied or expressed in these papers can have the weight of the more recent work on this subject. The examination of the works of Lang ('84), Kowalevsky ('83), Blochmann ('81), Rabl ('79), Bobretsky ('77), and Fol ('75, '76) has convinced me, however, that the principle of alternating spirals is of wide, if not universal, applicability among the various forms studied by these authors.

The number of cases in which the law is contradicted is surprisingly small. I shall deal with them more fully in my final paper, and for the present shall merely call attention to the nature of the more important contradictions. Some of them rest upon interpretations of the relations of cells which by implication or explicit statement of the author are conjectural, as in Kowalevsky's ('83) earlier and Metcalf's ('93) later work upon Chiton. Another kind of contradiction is found in two special cases: (1) in the condition leading to the eight-cell stage of Planorbis, — a form with sinistral shell, — as described by Rabl ('79, Taf. XXXII. Figs. 9, 10); and (2) in the corresponding stage of Janthina — a form with dextral shell (Fischer, '80–'87, p. 775) — according to Haddon ('82, Plate XXXI. Fig. 6). In both these cases, the spiral of the fourth generation, instead of being a right one, as in all other cases examined, is apparently a left one. These, however, do not necessarily present exceptions, for an alternation of spirals in successive generations may obtain even here, since the principle of alternation does not necessarily imply that the right spirals should in all forms give rise to the even generations. In other words, we may have in these two forms cases of "reversed cleavage." The decision of this point must be held in abeyance until further investigation can be made on these forms. Still other contradictions belong to a class which

ceases to be contradictory when the author's figures are relabelled, and thereby become better reconciled with each other as well as with the principle of the alternation of spirals. One such case is found in Lang's work on Discocœlis ('84, Taf. XXXV. Fig. 6), and another in Blochmann's paper on Neritina ('81). With the latter I shall deal in this paper. It is only fair to note that Blochmann's work was that of a pioneer in the field of cell lineage, and it is therefore not strange that later observers, in the light of comparative study, find their results at variance with his; as Wilson and Conklin have in reference to the origin of the "cross" (Wilson, '92, p. 441). I shall take great liberties with Blochmann's work, and shall endeavor to show that the cleavage of Neritina, as *figured* by him, conforms to the law of alternation of spirals, and shall give such arguments as I can from internal evidence and theoretical considerations to support my interpretation; but it is to be remembered that my conclusions remain hypothetical until verified or disproved by renewed observation. Figures 7–12 (Plate II.) are reproductions in outline respectively of Figures 45–48, 50, and 51 (Taf. VII.) of Blochmann's paper ('81), with *his* labelling outside the limits of the figures and *my own* inside the same limits. No exception is taken to his interpretation until the stage of Figure 10 is reached. Here I must differ radically from the author's interpretation of the relations of the two quartets a_3–d_3 and a_2^{I}–d_2^{I}. The designations of the cells a_3, d_2^{I}, d_3, c_2^{I}, c_3, b_2^{I}, b_3, a_2^{I}, must all be shifted one place to the right; i.e. in the direction of the hands of the watch, as indicated by the long arrows outside the limits of the figure in Figure 10. In my interpretation the quartet a_3–d_3 is the quartet designated as a_2^{I}–d_2^{I}, and *vice versa*. The change is made upon the following grounds.

(1.) The spindle in the cell b, for example, Figures 8, 9, indicates that the cell b_3 (Fig. 8, = my $d^{6.2}$) will lie above and to the *right* of the cell b (my $d^{6.1}$), and perhaps higher (Figs. 9, 10) than the upper derivative (my $d^{6.4}$) of the cell b_2 (my $d^{5.2}$), and that it will lie in contact with that derivative ($d^{6.4}$) and with the *lower* derivative ($a^{6.3}$) of the cell a_2 (my $a^{5.2}$). This is my interpretation of the position of the cell b_3, as foreshadowed by the spindles of Figures 8, 9. However, in Figure 10 Blochmann has placed the cell b_3 on the *left* side of the cell b, and in contact with a_2^{I} and b_2, a position directly contradictory to that indicated by the position of the spindle of the cell b in Figures 8, 9.

(2.) The spindle of the cell b_2 in Figures 8, 9, indicates that the products of its division will lie upon the upper left side of the cell b, the upper derivative being in contact with the cell b_3 and with the lower derivative. Blochmann's interpretation, on the other hand, throws the upper derivative completely out of the quadrant of the cell b, and over upon the cell c of an adjoining quadrant. The position of the cells a_3–d_3 and a_2^{I}–d_2^{I}, as figured by Blochmann in Figure 10 can be explained only on the assumption of a rotation of both sets of spindles (or of the upper derivatives of the spindles) as shown in Figure 9 about ninety degrees to the left. The fact that he has not observed such a rotation renders its existence all the more improbable.

There remains, however, important evidence in favor of Blochmann's view, namely the presence in the cells a_2 and c_2 of "eine Anhäufung von kleinen stark lichtbrechenden Körnchen." After the cell divisions indicated in Figures 46, 47 (Plate II. Figs. 8, 9), *all* of these granules are found in the cells a_2^{I} and c_2^{I}, hence the idea of their derivation from the cells a_2 and c_2. Such a view must presuppose (1) that in the equal division of the cells a_2 and c_2 all of these granules go to *one* of the supposed daughter cells, i. e. to a_2^{I} and c_2^{I}; (2) that granules similar to those of the cells a_2 and c_2 cannot arise in other cells derived, like a_2 and c_2, from the macromeres. If, on the other hand, the view advanced here as to the interpretation of Figure 48 (Plate II. Fig. 10) is correct, we may suppose that in the equal division of the cells a_2 and c_2 the granules accumulated in them were shared by the two daughter cells a_2 and a_3, c_2 and c_3 (using Blochmann's designations, but not his interpretation), and thus the granules were divided and became less conspicuous. At the same time the cells a_2^{I} and c_2^{I} of the third set of micromeres, originating, as I believe they do, from the macromeres b and d, show granules similar to those of the cells a_2 and c_2 of the second set of micromeres. This may afford an escape from the dilemma as to the relation of these groups of cells in which Blochmann is placed by the contradictory evidence afforded on the one hand by the position of the spindles, on the other by the presence of the granules in the cells.

With regard to the origin of the cells of the quartet a_2^{II}–d_2^{II} of Figure 50 (Plate II. Fig. 11), I wish to present a view which differs from that of the author. As the nomenclature indicates, he derives this group from his quartet a_2^{I}–d_2^{I} of Figure 48 (Plate

II. Fig. 10). There are indications, however, that they were really derived from the apical quartet a_1–d_1; for (1) their nuclei are nearer those of the apical quartet; (2) the cells of the apical quartet are much smaller after the cells a_2^{II}–d_2^{II} appear than before; (3) a_2^{I}–d_2^{I} have just arisen by a recent division, whereas some time has elapsed since the first division of the apical quartet, Figure 45 (Plate II. Fig. 7). If this interpretation holds, the cells a_1 and d_2^{II} result from the division of a_1, while b_1 and a_2^{II} come from b, etc. The inner part of the "cross" would thus arise from the first group of micromeres and the outer part from the third group. These suggested changes reduce the cleavage of Neritina to complete agreement with that of Nereis, Umbrella, and Limax.

Wilson ('92, p. 439) compares the cleavage of Nereis with that of the polyclad Discocœlis, and also with that of the gasteropods — taking Neritina and Crepidula as types — as follows: "Up to a late stage in the spiral period (twenty-eight cells) every individual blastomere and every cell division [in Nereis] is represented by a corresponding blastomere and a corresponding cell division in the embryo of the polyclad and in that of the gasteropod." This statement must imply, it seems to me, some other interpretation of the cleavage of Neritina than that given by Blochmann himself, although Wilson makes no statement to that effect, but on the contrary says (p. 442), "It is impossible to explain the differences between the annelidan and molluscan cross by assuming inaccuracy of observation on Blochmann's part, since the pole cells of the lateral arms show a peculiar granulation that may be seen in the parent cells ($a^{2.2}$, $c^{2.2}$) from which they rise." The reduction of Neritina to agreement with Nereis and Umbrella in the manner I have suggested brings it also into harmony with the law of alternation of spirals, and this affords another presumption in favor of the correctness of the revision here proposed. That the spirals do alternate in Neritina can be seen on an examination of the short arrows indicating my interpretation of the genetic relation of the cells of Neritina (Plate II.). The arrows show the relation of the cells, the head of the arrow in every case lying in the derivative which, according to my interpretation, is the upper one. The following table will assist in the comparison of the two interpretations and the determination of the spirals.

Revised.				Blochmann.
Spiral.	Nomenclature of Cells.	Generation.	Cell Stage.	Nomenclature of Cells.
Left . . .	a^3, b^3, c^3, d^3	3	4	a, b, c, d
Right . .	$\left\{ \genfrac{}{}{0pt}{}{a^{4.2}}{a^{4.1}} > a^3 \right\}$	4	8	$\genfrac{}{}{0pt}{}{a_1}{a} > a$
Left . . .	$\left\{ \genfrac{}{}{0pt}{}{a^{5.4}}{a^{5.3}} > a^{4.2} \right\}$	5	16	$\genfrac{}{}{0pt}{}{a_1}{a_1^{\mathrm{I}}} > a_1$
Left . . .	$\left\{ \genfrac{}{}{0pt}{}{a^{5.2}}{a^{5.1}} > a^{4.1} \right\}$		12	$\genfrac{}{}{0pt}{}{a_2}{a} > a$
Right . .	$\left\{ \genfrac{}{}{0pt}{}{a^{6.8}}{a^{6.7}} > a^{5.4} \right\}$	6	28	$\genfrac{}{}{0pt}{}{a_1}{d_2^{\mathrm{II}}} > a_1$
Not figured.				
Right . .	$\left\{ \genfrac{}{}{0pt}{}{a^{6.4}}{a^{6.3}} > a^{5.2} \right\}$		24	$\genfrac{}{}{0pt}{}{a_3}{a_2} > a_2$
Right . .	$\left\{ \genfrac{}{}{0pt}{}{a^{6.2}}{a^{6.1}} > a^{5.1} \right\}$		20	$\genfrac{}{}{0pt}{}{d_2^{\mathrm{I}}}{a} > a$
Left . . .	$\left\{ \genfrac{}{}{0pt}{}{a^{7.8}}{a^{7.7}} > a^{6.4} \right\}$	7	36	$\genfrac{}{}{0pt}{}{a_3}{d_3^{\mathrm{I}}} > a_3$
Left . . .	$\left\{ \genfrac{}{}{0pt}{}{a^{7.4}}{a^{7.3}} > a^{6.2} \right\}$		36	$\genfrac{}{}{0pt}{}{d_2^{\mathrm{III}}}{d_2^{\mathrm{I}}} > d_2^{\mathrm{I}}$

Only two spiral cleavages of the seventh generation are figured.

It will be noted that in Plate II. Fig. 10, the order in position of the quartets of the sixth generation taken from the vegetative toward the animal pole is as follows: $a^{6.1}-d^{6.1}$, $a^{6.3}-d^{6.3}$, $a^{6.4}-d^{6.4}$, and $a^{6.2}-d^{6.2}$, instead of $a^{6.1}-d^{6.1}$, $a^{6.2}-d^{6.2}$, $a^{6.3}-d^{6.3}$, and $a^{6.4}-d^{6.4}$, as in Limax, Plate I. Fig. 2. This change in the succession of quartets is due to the fact that in Neritina the quartet $a^{5.2}-d^{5.2}$ by virtue of the spiral occupies a position in the furrows between the macromeres $a^{5.1}-d^{5.1}$. Therefore, when the cleavages resulting in the sixth generation take place, the upper derivatives ($a^{6.2}-d^{6.2}$) of the quartet $a^{5.1}-d^{5.1}$ come to occupy a position nearer the animal pole than the upper derivatives ($a^{6.4}-d^{6.4}$) of the quartet $a^{5.2}-d^{5.2}$. The application of the system of nomenclature proposed in this paper is not thereby involved in difficulty, for the designation of the daughter cells is in all cases predetermined by that of the mother cell, according to the basis set forth on page 186, the upper derivative in every case receiving the even exponent and the lower one the odd. Thus, notwithstanding the secondary

changes induced in the position of the quartets by the accumulation of yolk in the basal quartet, the cells in Neritina retain the same nomenclature that is given to the homologous cells of a form such as Limax, presenting the typical undisturbed superposition of quartets.

In all the literature examined there occurs but a single case where the position of an indicated *spindle* contradicts the principle of alternation of spirals. This is found in Lang ('84, Taf. 34, Fig. 20) in the division of the cells ae_1-de_1, forming ae_1-de_1 and ae_6-de_6. Little can be said with regard to this except that we do not know the full nuclear history of this cleavage, and that it occurs at the close of the spiral period, immediately over the group of "Scheitelzellen" that has just sunk below the level of the remaining ectoderm. This may have produced secondary modifications in the direction of the spiral.

It remains for me to speak of the influence of yolk upon cleavage. Reference to Plate I. Fig. 2, shows that in Limax the cell $b^{5.8}$ belonging to the quartet of this generation, which is the last to divide, is a member of the *smallest* quartet of the fifth generation. It will further be seen that the order of the division of the quartets, viz. first, second, fourth, third, is also the order of size from largest to smallest, the quartet of largest cells, i. e. those presumably with the greatest amount of yolk being the first to divide in this generation, as it was also in the preceding generation. This phenomenon is by no means confined to Limax. It is found in a great variety of forms, and the greater the amount of the yolk the greater seems to be the tendency of the cells of the yolk-laden quartet to divide before those of the smaller quartets. This can be illustrated by a comparison of Figure 1 (Plate I.), which shows the egg of Limax approaching the sixteen-cell stage, and Figure 7 (Plate II.), which shows Neritina approaching the same stage. In Limax there is little yolk, and the cleavage of the two quartets is almost coincident. In Neritina, however, where the yolk is more abundant, the lower quartet has divided, and its nuclei have assumed a "resting condition" before the cleavage of the upper quartet has fairly begun. Thus we have a well marked twelve-cell stage. The same tendency of the yolk-laden cells to cleave before the others is seen in the later stages of Neritina, and indeed in many forms of Molluscan cleavage. It is also found in Nereis and Discocœlis. It is not, however, confined to the spiral

type of cleavage, for in Echinocyamus (Théel '92), where the cleavage is of the radial type, we find the meridional cleavages, which divide the quartets into "octets," starting with the fourth cleavage (eight- to sixteen-cell stage) at the vegetative pole, and reaching the apical quartet of the animal pole in the sixty-cell stage. Not only does the quartet having the largest cells divide first, but where there is an inequality in the size of the cells of a quartet, the largest cell or cells may divide first, as is the case in Discocœlis (Lang '84) and Unio (Lillie '93). In view of these facts I wish to raise the question as to the applicability to the individual cells of cleaving eggs of the law first formulated by Balfour ('75) and concisely expressed in his "Comparative Embryology" ('80, Vol. I. p. 95), as follows: "Where the yolk spherules are fewest, the active protoplasm is necessarily most concentrated, and we can lay down as a general law that the velocity of the segmentation in any part of the ovum is, roughly speaking, proportional to the concentration of the protoplasm there; and that the size of the segments is inversely proportional to the concentration of the protoplasm. Thus the segments produced from that part of an egg where the yolk spherules are most bulky, and where therefore the protoplasm is least concentrated, are larger than the remaining segments, and their formation proceeds more slowly."

It is true that, of two *eggs* otherwise similar, the one with the larger amount of yolk in general cleaves more slowly. For example, Umbrella, which has a large amount of yolk, requires four days to reach the stage attained by Limax in one day. But of two *cells* of Limax or Umbrella during the cleavage period, that one is the *first* to divide which is the larger and presumably has the greater amount of yolk, and which in Balfour's terms has its protoplasm less concentrated. Not only this, but in the cases cited the greater the amount of the yolk the greater is the tendency of the division of the yolk-laden cell to precede that of the cell with less yolk. There are, to be sure, many cases where Balfour's law actually applies, as in the frog's egg; but do not the cases cited above, belonging as they do to numerous and widely distributed classes of animals, form an important exception to the law as he has formulated it? A paradox is thus presented. Yolk appears to delay cleavage in the cells of the frog's egg, to hasten it in the cells of the snail's egg. Yolk also appears to delay the development of an organism as a whole (cf. Limax and

Umbrella) while it may (in Limax and Umbrella) at the same time apparently hasten the cleavage of those *cells* of the organism in which it is more abundant. This apparent conflict of statements has its foundation in the hypothesis that the amount of yolk alone is the decisive factor in the determination of the rapidity of cleavage. But there are other factors to be considered, especially the *quality* of both yolk and protoplasm, and in these there may ultimately be found some solution of the difficulty. In the case of the presumably undifferentiated blastomeres of the cleavage stages of Limax (and Umbrella) the difference in the rapidity of cleavage is apparently correlated with the greater or less absolute amount of protoplasm in the individual cells. The amount of protoplasm, in turn, is dependent on both the quality of the yolk and the activity of the protoplasm. The yolk, by contributing to the amount of protoplasm in the larger cells, may thus indirectly hasten their division. In this, however, the appropriation of the yolk by the protoplasm is the important factor, for in case the protoplasm fails to appropriate the yolk with sufficient rapidity, the division of the yolk-laden cell may be delayed as in the frog's egg. This delay may depend on either one or both of the two factors, — quality of protoplasm and quality of yolk. These same factors probably determine the differences in the rates of cleavage of different eggs. While this does not afford a solution of the difficulties encountered in attempting to harmonize the facts here presented with Balfour's law of cleavage as influenced by yolk, future inquiries in the direction suggested may lead to a better understanding of the factors determining the nature of cleavage.*

CAMBRIDGE, MASS., December 22, 1893.

---

* The substance of this paper was presented at the annual meeting of the American Morphological Society, in New Haven, Conn., December 29, 1893.

LITERATURE CITED.

Balfour, F. M.

'75. A Comparison of the Early Stages in the Development in Vertebrates. Quart. Jour. Micr. Sci., Vol. XVI. pp. 207–226, Pl. X. July, 1875.

'80. A Treatise on Comparative Embryology, Vol. I., xi + 492 + xxii pp. London, 1880.

Blochmann, F.

'81. Ueber die Entwicklung der Neritina fluviatilis, Müll. Zeitschr. f. wiss. Zool., Bd. XXXVI. pp. 125–174, Taf. VI.–VIII. 19. August, 1881.

Bobretsky, N.

'76. Studien über die Embryonale Entwicklung der Gasteropoden. Arch. f. mikr. Anat., Bd. XIII. pp. 95–169, Taf. VIII.–XIII. 1876.

Conklin, E. G.

'91. Preliminary Note on the Embryology of Crepidula fornicata and of Urosalpinx cinerea. Johns Hopkins Univ. Circ., Vol. X. No. 88, pp. 89, 90. May, 1891.

'92. The Cleavage of the Ovum in Crepidula fornicata. Zool. Anzeiger, Jahrg. XV. No. 391, pp. 185–188. 1892.

Fischer, P.

'80–87. Manuel de Conchyliologie et de Paléontologie conchyliologique ou Histoire naturelle des Mollusques vivants et fossiles. xxiv + 1569 pp., 23 Pl. Paris, 1880–87.

Fol, H.

'75. Études sur le développement des Mollusques. 1er Mémoire: Sur le développement des Ptéropodes. Arch. Zool. exp. et gen., Tom. IV. pp. 1–214, Pl. I.–X. 1875.

'76. Études sur le développement des Mollusques. 2me Mémoire: Sur le développement des Hétéropodes. Arch. Zool. exp. et gen., Tom. V. pp. 105–158, Pl. I.–IV. 1876.

Haddon, A. C.

'82. Notes on the Development of Mollusca. Quart. Jour. Micr. Sci., Vol. XXII. pp. 367–370, Pl. XXXI. 1882.

Heymons, R.

'93. Zur Entwicklungsgeschichte von Umbrella mediterranea, Lam. Zeitschr. f. wiss. Zool., Bd. LVI. pp. 245–298, Taf. XIV.–XVI. 1893.

Kowalevsky, A.

'83. Embryogénie du Chiton Polii (Phillippii), avec quelques Remarques sur le Développement des autres Chitons. Ann. Musée

Hist. Nat. Marseille, Zool., Tom. I., Mém. No. 7, 55 pp., 8 Pl. 1883.

Lang, A.

'84. Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie. Fauna u. Flora d. Golfes v. Neapel, XI., ix + 688 pp., 54 Holzschn., Atlas 39 Taf. Leipzig, 1884.

Lillie, F. R.

'93. Preliminary Account of the Embryology of Unio complanata. Jour. Morph., Vol. VIII. pp. 569–578, Pl. XXVIII.

Metcalf, M. M.

'93. Contributions to the Embryology of Chiton. Studies Biol. Lab. Johns Hopkins Univ., Vol. V. No. 4, pp. 249–267, Pl. XV., XVI.

Rabl, C.

'79. Ueber der Entwicklung der Tellerschnecke. Morph. Jahrbuch, Bd. V. Heft 4, pp. 562–655, Taf. XXXII.–XXXVIII., 7 Holzsch. 1879.

Théel, H.

'92. On the Development of Echinocyamus pusillus (O. F. Müller). Nova Acta Reg. Soc. Sc. Upsala, Ser. III. Vol. VI., 57 pp., Pl. 9. 1892.

Wilson, E. B.

'92. The Cell Lineage of Nereis. A Contribution to the Cytogeny of the Annelid Body. Jour. Morph., Vol VI. No. 3, pp. 361–480, Pl. XIII.–XX.

'93. Amphioxus and the Mosaic Theory of Development. Jour. Morph., Vol. VIII. No. 3, pp. 579–638, Pl. XXIX.–XXXVIII.

EXPLANATION OF PLATE I.

Figures 1–4, Limax, drawn with camera lucida.

Figure 1. Eight-cell stage from anterior end, × 375; *p.g.*, polar globules; *s.c.*, segmentation cavity.

" 2. Twenty-four-cell stage from right side, × 375.

" 3. Twenty-five-cell stage from the animal pole, × 375.

" 4. Same egg from vegetative pole, × 375.

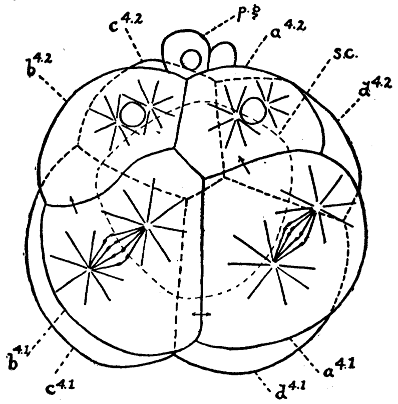
Figures 5, 6, Nereis after Wilson ('92).

" 5 After Plate XIV. Fig. 21, "Rear view of twenty-two-cell stage. Division of X, spindles of c^3, d^3."

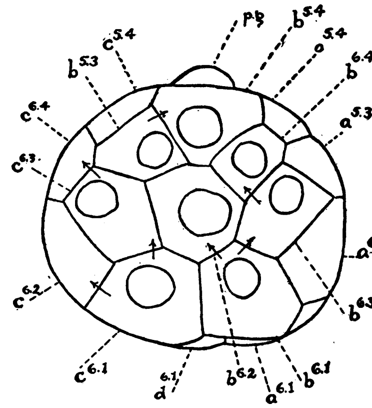
" 6. After Plate XV. Fig. 25, "Thirty-two- (four-) cell stage, right side-view. Third spiral cleavage of a^1, b^1, c^1, d^1, in progress."

PLATE I.

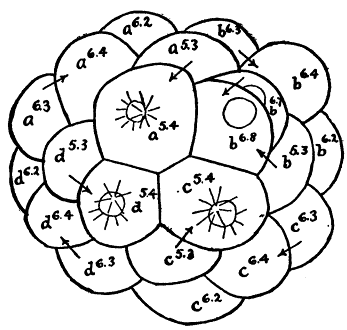
1.



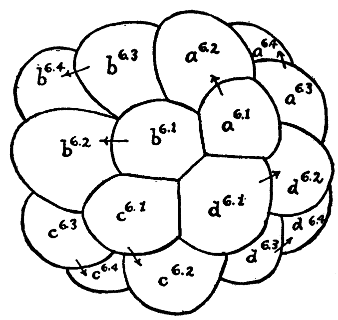
2.



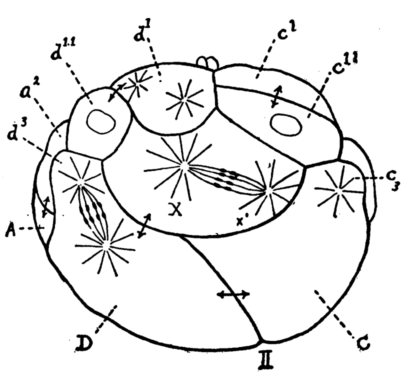
3.



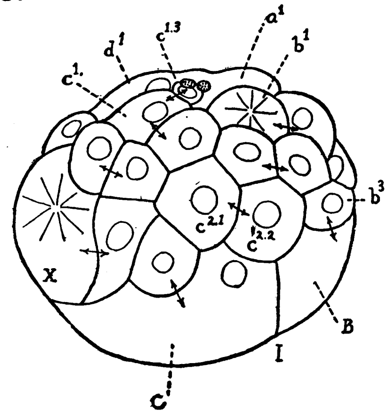
4.



5.



6.



EXPLANATION OF PLATE II.

Neritina after Blochmann ('81).

The arrows, indicating the derivation of cells, and the nomenclature placed within the limits of the figures are mine; the rest of the nomenclature is reproduced from Blochmann's figures. It is to be observed that his lettering, *a, b, c, d*, is from right to left, whereas my own is that generally accepted, — from left to right. The explanation of these figures is taken from Blochmann, and the term "generation" is used in the sense in which he has employed it.

Figure 7. Taf. VII. Fig. 45. "Formation of the cells of the sixth generation, a_1^{I} b_1^{I} c_1^{I} d_1^{I}. *Rbl*, polar globules."

" 8. Taf. VII. Fig. 46. "Formation of the cells of the seventh and eighth generations, a_3 b_3 c_3 d_3 and a_2^{I} b_2^{I} c_2^{I} d_2^{I}."

" 9. Taf. VII. Fig. 47. "The same stage in profile."

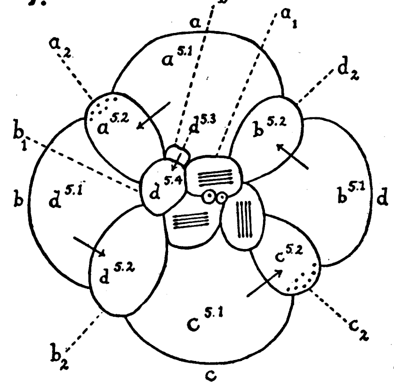
" 10. Taf. VII. Fig. 48. "Completed twenty-four-cell stage." The long arrows, indicating a change of interpretation, are mine.

" 11. Taf. VII. Fig. 50. "Twenty-eight-cell stage; a_2^{II} b_2^{II} c_2^{II} d_2^{II}, cells of the ninth generation."

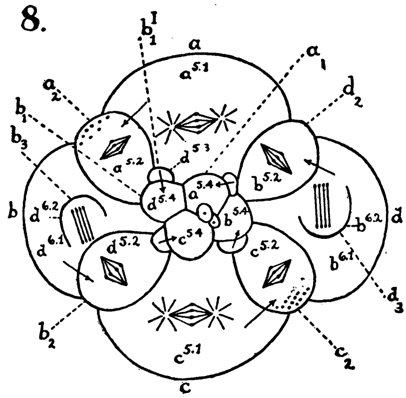
" 12. Taf. VII. Fig. 51. "Thirty-six-cell stage; *vz* derived from a_2^{II}, vz_1 from c_2^{I} [my $b^{7.8}$], d_2^{III} from d_2^{I}, a_3^{I} from a_3, etc.

PLATE II.

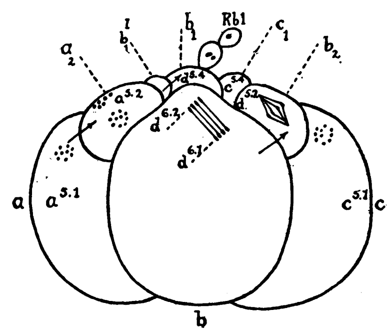
7.



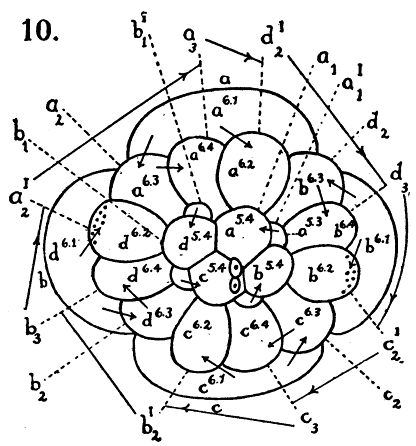
8.



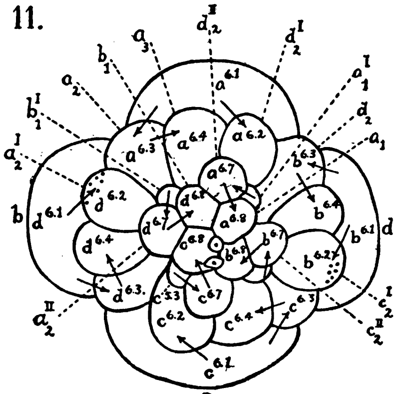
9.



10.



11.



12.